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THE EFFECT OF 2,450 MEGAHERTZ MICROWAVES ON THE SURVIVAL OF *BACILLUS GLOBIGII* SPORES

A THESIS

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THE EFFECT OF 2,450 MEGAHERTZ MICROWAVES ON

THE SURVIVAL OF *BACILLUS GLOBIGII* SPORES

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SUMMARY

The possibility of contaminating extraterrestrial objects in increased if probes from earth are not sterile. A very important aspect of this problem is the presence of sequestered bacterial spores in probe components. In this study an effort was made to approximate the physiological state of the sequestered organisms by working with dried preparations in order to evaluate a previously untested method, irradiation with microwaves, for decreasing the viability of these spores. Radiation with a frequency of 2.450 Gigahertz (wavelength of 12.25 cm) was focused onto an air-dried sample of Bacillus globigii spores. The power density was controllable. The temperature at the surface of the target remained ambient. The percentage of surviving organisms remained above 90% for all power densities up to 1.19×10^{11} erg/cm^2. This study provides evidence that energy at the frequency of microwave ovens is ineffective in the decontamination of air-dried samples of spores.

CHAPTER I

INTRODUCTION

Interest in maintaining planetary quarantine -- that is, preventing extraterrestrial contamination -- stems from the possibility that life on one planet may not be compatible with life on another (1). Furthermore, contamination of an extraterrestrial body may eliminate any chance of detecting life which has originated on that planet.

One aspect of the problem of planetary quarantine involves the sterilization of unmanned space probes. Some of these probes are designed to orbit planets, some are designed to by-pass planets, and some are designed to land on the planetary surface. It is possible that, in landing, the instrument package will be shattered on impact - resulting in the exposure of the interior of the probe to the new environment. Therefore, the interior of the probe should be sterile. The following example illustrates the difficulties involved in sterilization. Portner (2) tested the interior of electrical components such as ceramic capacitors, resistors, and transformers for microbial contamination; she reported that sequestered organisms survive the process of construction of the components.

Electromagnetic energy of various frequencies has been used in attempted sterilization procedures. Infrared electromagnetic radiation, generating heat, is often used for this purpose (3,4,5). Ultraviolet radiation has also been widely used for decontamination of surfaces (5,6, 7). Electromagnetic radiations at wavelengths longer than those in the

infrared spectrum are known as microwaves. These wavelengths are generally in the range of 1 m to 10^{-3}m; although this radiation possesses less energy than infrared or ultraviolet, the ability of microwaves to penetrate certain materials is much greater than that of the other two kinds of radiation (8,9,10,11).

Microwaves are extremely effective in heating dielectrics*. Internal heating occurs because the oscillating electromagnetic field of the radiation causes molecules to oscillate back and forth synchronously with the radiation field; friction between the driven molecule and its neighbors generates heat. The molecule oscillates with the field because it possesses a non-symmetrical electrical charge distribution, either inherent or induced during the irradiation, which responds to the electric field of the microwave radiation.

Water is a dielectric, and is, therefore, very well suited for absorbing microwave energies. The molecule is not large; at ambient temperatures it moves about rather freely; it has a dipole moment of 1.8 D**; and it is known to absorb microwaves (10). Thus, water is an important molecule with respect to microwave irradiation.

Several investigators have used microwaves in studying biological phenomena. A few of these studies are discussed below.

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*Materials which are not good electrical conductors may be considered dielectric materials.

**The dipole moment is a measure of the charge asymmetry of the molecules, a dipole consisting of an electron separated from a unit positive charge by a distance of 1Å would have a dipole moment of $(4.8 \times 10^{-10}\text{esu})\ (10^{-8}\text{ cm}) = 4.8 \times 10^{-18}$ esu cm or 4.8 Debye Units.

Lechowich et al. (12) irradiated six ml suspensions of Streptococcus faecalis and Saccharomyces cerevisae at concentrations of 10^8 to 10^9 per ml with 12.25 cm microwaves. The samples were continuously cooled by kerosene at -25°C. By comparing survivor curves of convection-heated suspensions with survivor curves of microwave-heated suspensions lethality was reported to be due to thermal effects.

Goldblith and Wang exposed aqueous suspensions of E. coli and B. subtilis cells to 12.25 cm radiation and compared the results to the effects of heating the samples. They observed a six decade reduction in viability at 100°C. Microwave heating and conventional heating survival curves were identical (13).

Although specific frequency, power levels incident on the target, dimensions of the target, and period of exposure were not mentioned in a report by Tomberg (14), a few interesting findings are restated here. Due to the thermal action of high frequency fields, the following information was summarized: (a) permeability between cells increased more due to irradiation than due to the direct application of heat; (b) growth of microbes or plant seeds was stimulated while the temperature increase was negligible; (c) growth of microbes and plant seeds was inhibited at temperatures normally associated with optimal growing conditions. The method of temperature measurement and specific organisms used were not mentioned.

Silverman (15), in summary, mentions that work on the effect of microwaves above 3 GHz on microorganisms has not been attempted. He also indicates the type of problems to which this may be applied, such as irradiation of bacterial spores in the dry state with electromagnetic energy to reduce their activity.

Some other microwave work has centered on irradiation of mammalian tissues (8,9,16,17) and on whole-body irradiation of mammals (18,19). The relative absorption cross section of tissues to microwaves of various wavelengths has been studied (9). The investigation correlated theoretical calculations with experimental observations. This report centered around biological targets much larger than the wavelength of the incident microwaves which is not the case when illuminating bacteria with 12.25 cm microwaves, and therefore, is not directly applicable. Experiments involving microwave illumination that does not result in "evident pathology" was studied (20). Electrical properties of mammalian tissue, including reflection and absorption, were considered. The possible explanation of affects includes anomalous dispersion and molecular relaxation times (14). The formation of free radicals (oxygen and hydroxyl groups) at "high power levels" was suggested, however, the mechanism of their formation was not mentioned.

The majority of the work reported, involving the interaction of biological materials and microwaves often does not include an in-depth discussion of physical parameters. The recent increase in interdisciplinary efforts of biologists and engineers will undoubtedly elucidate the biological effects of such factors as dielectric constants, incident power density vs. power density absorbed, and wavelength of illumination. It is primarily because of the omission of the discussion of such possibly relevant biological and electrical factors that some of the literature on biological effects of microwave radiation is less meaningful than it might be.

To date there is no information on the effect of 12.25 cm wave-

length radiation on microorganisms that might be found deep inside electrical components. As mentioned earlier, this may be a critical consideration in the quarantine problem. Thus far, the only microorganisms which have been found to be sequestered in such hardware are bacterial endospores. Spores contain water; thus, I am interested in dielectric heating of water with respect to spore survival. Water moves freely in and out of the bacterial spore; in fact, the interior of the spore has the same relative humidity as its environment at equilibrium unless the relative humidity of the environment is less than the concentration of bound water in the spore (21). Any residual water in a spore should respond to 12.25 cm radiation. At sufficiently high water concentration and microwave intensity, the spore should be killed due to the heating of the water which the spore contains.

CHAPTER II

MATERIALS AND METHODS

Organism Used

A Bacillus globigii spore suspension was obtained from the laboratory of Dr. Peter Skaliy, Microbiological Control Section, Epidemiology Program, Bacterial Diseases Branch, National Center for Disease Control, Atlanta, Georgia. This suspension was plated on Difco thermoacidurans agar modified (TAM) medium. To insure purity of the strain, the resultant growth was streaked five times, at 24 hour intervals, and an isolated colony picked at each streaking for inoculation of the next plates.

Characterization Studies

The final pure strain was characterized, biochemically and morphologically, and compared to the description in Miscellaneous Publication 559, USDA, Aerobic Mesophilic Spore Forming Bacteria, pages 56-58, 66-67, as shown in Table 1.

Production of Stock Spore Suspensions

Twelve plates of solidified TAM medium were inoculated over their entire surfaces with 0.1 ml of a suspension containing Bacillus globigii, using a sterile, bent-glass (150°) rod and incubated at 37°C for five days. The resultant growth was scraped off with a sterile glass rod and diluted in 100 ml of Sorenson's phosphate buffer (pH 6.9). Subsequently, this suspension was heated for 15 minutes at 80°C to kill any viable vegetative cells that were present. Using the pour-plate technique,

Table 1. Biochemical Characterization

	B. globigii	Test Organism
Nutrient agar slants	+	+
Glucose nitrite agar slants	++	+
Tyrosine agar-substrate blackened	+	+
Nutrient broth-clear broth with pellicle	+	+
Voges-Proskaur	+	+
Citrate	+	+
Nitrites from nitrates	+	+
Casein hydrolysis	+	+
Gelatin hydrolysis	+	+
Starch hydrolysis	+	+
Lactose utilization	-	-
Maximum temperature for growth	50-54° C	50-54° C
Maltose	+	+
Xylose	+	+
Raffinose	+	+
Galactose	+	+
Arabinose	+	+
Sucrose	+	+
Mannose	+	+
Salicin	+	+
Glucose	+	+
Glycerol	+	+
Inulin	+	+
Fructose	+	+
Potato broth-heavy growth	+	+
Wrinkled pellicle over surface	+	+
White, yellow, pink, or brown, brown on aging	+	+

dilutions of 10^{-6} through 10^{-13} were inoculated in five TAM plates each (forty plates total). These plates were incubated at 37°C for 18 hours.

The resulting plate counts were used to bring the final concentration of spores in the 99 ml stock suspension of 10^7 organisms per ml by further dilution with phosphate buffer (pH 7.1). The diluted stock was put into one hundred four 16 x 125 mm screw-cap tubes, one ml per tube, and stored at - 10°C.

Irradiation Source

The irradiation apparatus was specifically designed (22) to allow microwave illumination of small samples at a wavelength of 12.25 cm. The power density was controllable at any arbitrary power level up to 1 Watt/cm^2 within 1 decibel accuracy. A Litton 1-5001 continuous wave magnetron was used to generate the microwaves.

The illuminator used in this research was a 1.17-meter diameter focused, elliptical antenna. The energy was fed to the antenna at one of its foci and the sample was placed at the other end. It was illuminated by a choked open-ended waveguide at one focus. See Figure 1.

Irradiation Procedure

Microscope slides were washed in 90% ethanol and rinsed in distilled water. A paper clip and slide were placed inside a 100 ml, wide-mouth specimen jar. The screw-cap was loosely fastened and the jar, including its contents, was autoclaved at 121°C for twenty minutes. The jar was allowed to cool to room temperature. The paper clip was then used to hook the slide to a string inside a modified decending-chromatograph chamber for irradiation.

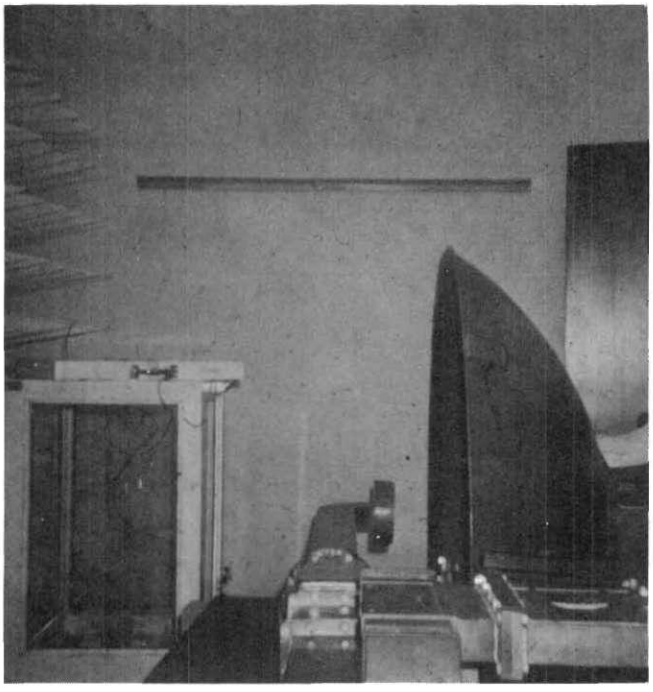

Figure 1. Antenna and Target Housing

Each experimental run was performed using a different sample of the frozen spore stock. The spore stock was assumed to be morphologically homogeneous. Tubes containing one ml of frozen stock spore suspension were selected arbitrarily and allowed to thaw to room temperature. Nine ml of sterile, distilled water was added aseptically to each tube to bring the concentration of cells to 7.5×10^6 spores per ml. A 0.1 ml pipette was used to deposit 0.01 ml of the spore suspension on the precleaned, sterile microscope slides. This resulted in a target about 1 cm in diameter which contained 7.5×10^4 spores.

The spores were deposited on the slides as follows: A cooled, sterile jar containing a paper clip and horizontal microscope slide was placed on its side on paper towels in an aluminum pan. The loosely fastened cap was partially removed and 0.01 ml of the spore suspension was deposited in the center of the slide. The jar cap was taped on only at one point, so that air could reach the inoculated slide. A piece of aluminum foil was placed over the pan containing the jars. The spores were allowed to air-dry on the slides for 8 to 16 hours. The jars were then tightly sealed, using screw-caps, and taken to the irradiation facility while still in the pan.

While the power supply was warming up, which took about five minutes, a modified-chromatograph-drying chamber, which was used to house the inoculated slides during irradiation, was moved into place. The chamber consisted of a wooden frame ($15\frac{1}{2}$" x $15\frac{1}{2}$" x 27") supporting four plate-glass panels (12" x 12" x 20") each. The glass panels were positioned perpendicular to the direction of propagation of the microwaves. At the top of the chamber a piece of string was taped across the width

of the inside chamber perpendicular to the incident waves. From this string three stainless steel hooks ($\frac{1}{2}$" in length) were hung. One side of the hook was placed over the string, and one side was left hanging in order to hold a slide by its paper clip (see Figure 2). The slide was positioned so that the inoculated surface of the microscope slide faced the focusing antenna and was, thus, parallel to the glass on the front of the drying chamber. The slides were handled with 8" sterile forceps.

The interior of the chromatograph-drying chamber was kept dry by placing a bag of activated Eagle Gel-C Dessicant* inside the housing 30 minutes prior to each illumination. The power density was controlled by means of a variable directional coupler which is described in detail by Boggs (22).

After the microscope slides were in place, the power density set, and the power supply warmed up, high voltage was applied to the magnetron in order to generate microwaves.

The unirradiated controls were inoculated slides placed near the bottom of the irradiation chamber in the same position as the irradiated slides. The power density in this area was found to be less than the smallest amount measurable by a radio frequency probe**(22).

Post-irradiation Treatment of Irradiated Samples

After the desired irradiation dose was administered, the high voltage to the magnetron was turned off. The slides were removed from

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*Eagle Chemical Co., Mobile, Alabama, 8 units, (MIL-D-3464-D).

**Narda Microline Electromagnetic Radiation Monitor, Model 8100, The Narda Microwave Corporation, Plainview, New York.

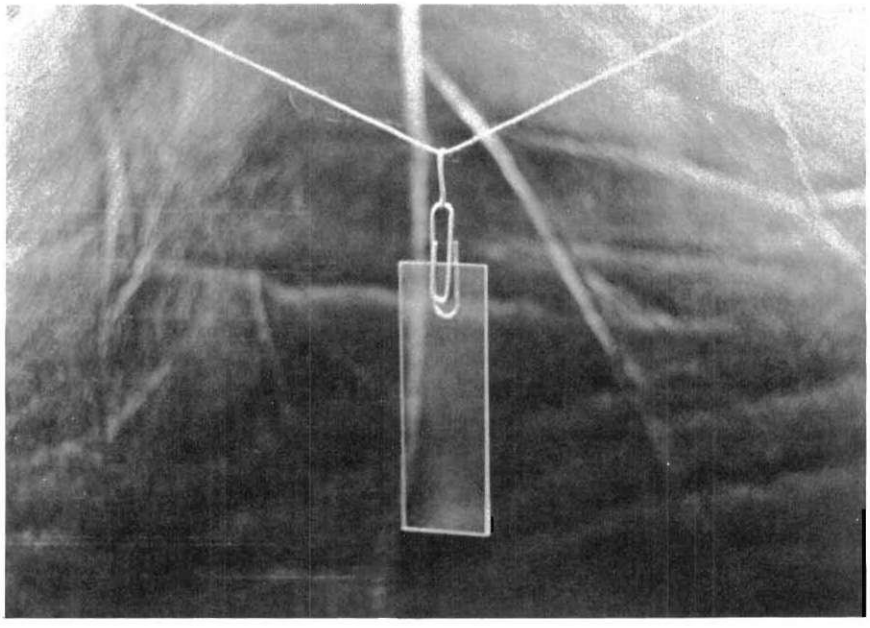

Figure 2. Target Support

the chamber with 8" sterile forceps and placed in their respective specimen jars. Within thirty minutes after irradiation, fifty ml of BBL Trypticase Soy Broth containing 1.0% Tween 80* was added to each specimen jar and the jars were placed on a rotary agitator at 120 rpm for ten minutes to wash the spores off the microscope slides. Tween 80 was added to the trypticase soy broth as a surfactant to aid in freeing the spores from the surface of the microscope slide.

Using a graduated 1 ml pipette, 0.1 ml aliquots of each broth washing were placed in the bottom of each of three plastic petri plates. BBL Trypticase Soy Agar at 40° - 45°C was poured into each plate and the plates were gently swirled to insure uniform mixing of organisms in the medium. The plates were allowed to solidify at room temperature. They were taped together, placed in a plastic bag, and incubated at 37°C for 24 hours. At the end of the incubation period, the plates were removed from the incubator, the colonies were counted using a Bactronic colony counter, and the results were recorded. Counts from the controls were compared with counts of the illuminated slide cultures to determine the change in survival due to the incident microwave energy.

Temperature Measurements

A copper-constantan, steel-jacketed thermocouple was used in conjunction with a temperature potentiometer**, a Bausch and Lomb 10 mv VOM 5

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*Polyoxyethylene (20) Surbitan Monooleate, Fisher Scientific Co., (#T-164), Atlanta, Georgia.

**Leeds and Northrup Company, Philadelphia, Penn., catalogue number 8695.

Recorder, and a 10 X solid-state operational amplifier* to monitor the temperature at the surface of the microscope slides subsequent to illumination and, in one case described below, to monitor the change in temperature during illumination of a model system.

During four preliminary runs, the temperature measuring equipment was connected and as the magnetron was turned off, the temperature at the surface of the irradiated slide was measured. The time response of the thermocouple was found to be less than $\frac{1}{2}$ second. During two temperature-measuring experiments, temperature response was recorded while the thermocouple was being illuminated. One temperature measurement was performed using a model system. Powdered glycine was placed on the tip of the thermocouple with saliva. The tip of the thermocouple was then irradiated for two hours at 200 mw/cm^2 and the temperature was recorded. This system was used in an attempt to simulate conditions at the target during illumination. The glycine plus saliva were intended to simulate air-dried spores, since the spores contain little moisture and are primarily protein.

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*Designed and constructed by Mr. Karl Branch, Microbiological Control Section Electronics Shop, National Center for Disease Control, Atlanta, Georgia.

CHAPTER III

RESULTS

The concentration in each tube of stock spore suspension was intended to be 7.5×10^7 spores per ml. The aliquot of this suspension placed on each microscope slide was 0.001 ml, which corresponds to 7.5×10^4 spores per microscope slide. On washing in 50 ml of trypticase soy broth with 1.0% Tween 80, the concentration was further diluted to 1.5×10^3 spores/ml. Plating out a 0.1 ml sample of broth washing should give a final concentration of 150 spores per plate. It was found that the concentration of spores was not the same in all stock tubes, due to inaccuracy in the dispensing technique. This caused the absolute number of colonies per agar plate to vary from experiment to experiment but had no effect on the relative number of colonies when control plates were compared to test plates of the same experiment. While the actual plate counts ranged from 65 to 219 for all experiments, collectively in each experiment plate counts varied 10% or less. Survival was defined as the ability to reproduce. Survival was measured by counting colonies on each agar plate after incubation. The data are shown in Table 2 and Figure 3. The statistical treatment is given in Appendix B.

Table 2. Incident Energy, Dose Rate, and Survival

Energy Incident in ergs/cm^2 x 10^{-10}	Actual Plate Counts	Mean Plate Count	Percent Survival	Dose Rate (Hrs @ mW/cm^2)	
1.44	83, 90, 89	87.33	97.3	2.0	200
1.44	92, 84, 96	90.67	89.9	2.0	200
0.00	93, 84, 106	94.33	100.0	0.0	0.0
1.62	74, 64, 64	67.33	91.5	2.0	800
1.62	81, 78, 75	78.00	94.2	2.0	800
1.62	80, 79, 75	78.00	94.2	2.0	800
0.00	77, 80, 83	80.00	100.0	0.0	0.0
2.00	142, 151, 156	149.67	90.3	2.78	200
2.00	161, 160, 173	164.67	99.1	2.78	200
0.00	160, 165, 172	165.67	100.0	0.0	0.0
2.68	135, 143, 132	136.67	99.9	3.0	200
2.68	141, 115, 125	127.00	93.4	3.0	200
0.00	135, 132, 145	137.30	100.0	0.0	0.0
3.50	108, 105, 118	110.30	100.0	3.0	325
3.50	112, 109, 102	107.67	97.6	3.0	325
0.00	104, 117, 110	110.30	100.0	0.0	0.0
3.99	111, 105, 102	106.00	96.1	3.42	325
3.99	106, 110, 97	104.30	94.6	3.42	325
0.00	104, 117, 110	110.30	100.0	0.0	0.0
4.32	176, 158, 170	168.00	91.4	3.0	400
4.32	185, 183, 219	195.67	104.1	3.0	400
4.32	164, 180, 176	173.33	94.1	3.0	400
0.0	165, 187, 202	184.67	100.0	0.0	0.0
11.98	139, 145, 130	138.00	103.2	8.28	400
11.98	147, 140, 133	140.00	104.6	8.28	400
0.00	135, 139, 127	133.67	100.0	0.0	0.0

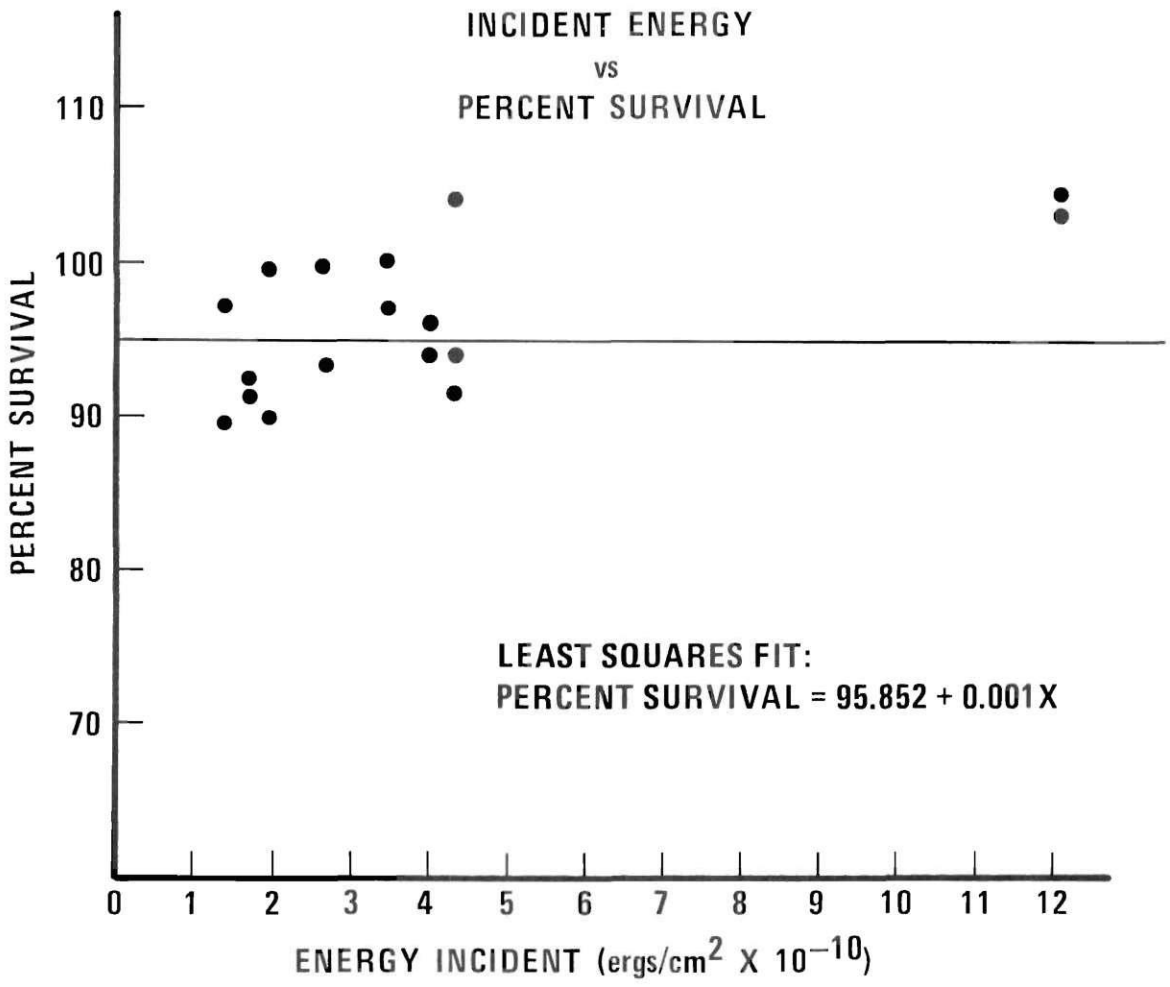

Figure 3. Results

Table 3. Analysis of Data*

Mean Percent Survival, $\bar{X}$	96.41
Variance, S^2	21.33
Standard Deviation, S	4.62
Standard Error, $\sigma_{\bar{x}}$	1.09
Number of Spore Samples Illuminated**, N	18.0
Correlation Coefficient, r	0.6458
Correlation Constants,	
a	94.852
b	0.0012

*Statistical Analysis Performed using Programs 2.14 and 2.16 of the Olivetti Programma 101 Library of Statistical Analysis.

**Three washings from each of these illuminations were analyzed for colony-producing ability.

CHAPTER IV

DISCUSSION

Substances which have the ability to dissipate incident electromagnetic energy as heat are referred to as "lossy" materials. The more lossy the substance, the hotter it becomes when illuminated with microwaves (23). A method of measuring this property is by observing its dielectric constant and the loss tangent. The loss tangent is proportional to the ratio of the power lost in heat (dielectric heat loss) to the energy stored per cycle, and thus, is a good measure of how lossy a dielectric material is. The higher a dielectric constant and the loss tangent, the more lossy it is, and consequently, the hotter it becomes when illuminated (23). Water has a characteristic dielectric constant of 78.54 at 25°C, which is high and dried protein has a characteristic dielectric constant of about 3 (9), and a loss tangent of about 0.3 (22).

At 2.450 GHz (a wavelength of 12.25 cm) the majority of energy absorption in the spore is accomplished by water molecules. This is suggested since water is a small molecule with a large dipole moment and requires less energy for the rotation peculiar to molecular dipoles, called forced oscillation.

In general, molecules may be affected by microwaves in two ways. The first method involves an incident electromagnetic wave causing a molecule with a permanent electric dipole moment to attempt to align its asymmetric charge with the oscillating field. The forced oscillation of a molecule has an effect on its neighbors. The movement creates

friction* if the molecules are close enough to rub against each other. The movement of the molecule in the field is best examined from a classical approach, that is, on a macroscopic level. If the microwave field is turned off, the molecule will stop rotating, and assume a random orientation. The energy transferred from the molecule that was induced to rotate, to its neighbor, by friction, is somehow accumulated. The accumulation of energy results in an event which requires more energy than is associated with microwaves. The accumulation of energy may induce nuclear vibrations in covalently bonded atoms. When the frequency of these vibrations is high enough, energy may be lost from the vibrating nuclei as light. The wavelength of the emitted light classifies it as infrared, and it is detectable as heat.

The second effect of microwaves on molecules involves quantized energy states. Molecules preferentially absorb characteristic wavelengths. The preference involves the energy associated with the wavelength and the energy required to promote the molecule to a state of higher energy. In this case the higher energy state involves rotational energy. If the incident microwave field is turned off, the molecule continues to rotate for a while. There has been energy absorbed directly by the molecule from the microwave field. Eventually this energy will be reemitted and the molecule returns to its ground rotational state.

Calculations were performed with respect to the energy associated with the wavelength of 12.25 cm and the energy required to induce rotation in the water molecules, which are believed to be the primary

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*Friction, as used here, is meant to describe a classical effect of heat generated by rubbing two objects together.

absorbers at approximately 10^{11} Hertz. Even though the oscillation time was long enough to cause rotation and, therefore, to increase temperature, no temperature change was indicated by thermocouple, nor was there sufficient heat to affect the survival of the spore. Based on these observations, one might draw one of several conclusions: (a) the water is not free to rotate, i.e., possibly bound to proteins or nucleic acids; (b) the number of water molecules induced to rotation was insufficient to permit detection; or (c) the undetectable temperature increase occurred in a noncritical area of the cortex, rather than in the more sensitive area containing the genetic material.

The water content of the spores was estimated to be ten percent, and the spores were assumed to be one-micron cubes. However, even if the moisture content of each spore were one hundred percent, the overall effect of the microwaves would be about the same. This observation is based on calculations performed using the expression $E = h\nu$, where E is the energy per quantum; h is Planck's constant, 6.63×10^{-27} erg sec.; ν is the frequency in Hertz. The dielectric relaxation time of water molecules is about 10^{-11} seconds, which corresponds to a frequency of 10^{11} Hertz. The energy required for maximum rotation of water molecules, and thus for the maximum heat production, is

$$E = h\nu$$

$$E = (6.63 \times 10^{-27} \text{ erg sec})\ (10^{11}/\text{sec})$$

$$E = 6.63 \times 10^{-16} \text{ erg/quantum} \quad \text{or}$$

$$(6.63 \times 10^{-16} \text{ erg/quantum})(6.2 \times 10^{11} \text{ ev/erg}) \cong 10^{-5} \text{ ev/quantum.}$$

The energy supplied by the 12.25 cm microwaves per quantum was

$E = h\nu$

$E = (6.63 \times 10^{-27} \text{ erg sec})(10^{9}/\text{sec})$ or

about $(10^{-18}$ erg sec$)(10^{11}$ ev/erg$) = 10^{-7}$ ev/quantum. The difference in energy required to rotate an unrestrained water molecule and that supplied by the incident microwaves is about 10^{2} ev/quantum. Thus 12.25 cm radiation can rotate unbound water molecules -- but not at the maximum possible rate. If the water is restrained, the difference would be even greater. In bacterial spores the water probably is bound to some proteins and nucleic acids.

This test system suggests a method for determining the amount of bound water in a cell. The bacterial spore is not selectively permeable to water (21). At equilibrium, the moisture content of the spore will be the same as the moisture content of the air around it, assuming that the water content of the air exceeds the concentration of bound water in the spore. Water absorbs electromagnetic energy of wavelengths in the general range of 15 cm to 1 cm very well, with absorption increasing as the wavelength approaches 1 cm (24). Rotation induced by the oscillating electromagnetic field causes water molecules to rub against neighboring molecules and the resulting friction causes a rise in temperature. Such temperature increases should be measured using an infrared detector. The wavelength necessary to cause induced rotation of free water molecules may be calculated (see preceding paragraph). The energy required above the level which was calculated to be sufficient to cause a temperature change, corresponds to the energy which is being used to restrain the water molecules. By estimating the number of water molecules in a spore,

knowing the wavelength of the radiation, and calculating the energy required to restrain the water molecules from rotation, the concentration of bound water in a bacterial spore may be, in principle, determined.

If the spore is sequestered in a material whose dielectric constant is much higher than that of the spore, illumination by the microwaves produces a much greater effect. As an example, suppose that a steak is to be cooked in a microwave oven. The meat is placed on a plastic wrapper while in the oven. The power is turned on and heat is generated, primarily as a result of the water molecules oscillating in the electromagnetic field. The heat denatures the proteins in the steak and the proteins in the spores (or germinated cells) which are inside the steak. The microorganisms which were resting on the plastic and not in the steak are not heated and, therefore, are not affected because there is not enough water present in the microorganisms to absorb sufficient energy to cause heat production. If the steak subsequently is stored in the plastic wrap, the unaffected organisms surviving on its surface will contaminate the meat.

CHAPTER V

CONCLUSIONS

No detectable effect on the survival of air-dried Bacillus globigii spores is elicited by 12.25 cm electromagnetic energy, at the power-levels tested, over reasonable time intervals. There may be several reasons for this effect.

The ratio of surface area to volume of the spore could be important in heat dissipation. There could be some heating inside the microorganism but the rapid dissipation would make it extremely difficult to detect. If the Bacillus globigii spore were thermally insulated during illumination, the dissipation might be minimized and, consequently, the effective heating might be enhanced.

APPENDICES

APPENDIX A

Maximum Possible Temperature Rise

The following calculations estimate the maximum possible temperature rise per spore. The following assumptions must be made:

(1) The water absorbed all of the energy incident on the spore.

(2) The water molecules were free to rotate; they were not bound.

(3) The water converted all of the incident energy to heat.

(4) The water in the spore retained all of the heat.

Based on these assumptions, the following order of magnitude approximation gives an estimate of the greatest possible increase in temperature by a bacterial spore.

Although the bacterial spore is a complex chemical system, the most important component with respect to microwave absorption at about 12 cm wavelength is water. A one-centimeter layer of human blood plasma, contained in a rectangular glass cuvette, absorbs about 25% of the incident energy at 2,450 megaHertz (22). The value of 25% is based on extensive calculations which consider reflection and refraction interactions at the air-glass, glass-plasma, and plasma-glass interfaces. In the irradiation of these Bacillus globigii spores, there is no plasma-glass or glass-plasma interface, and the biological target is a thin film on the glass microscope slide, not plasma. Important similarities in the two systems are that they both contain water, and that they both have a

target-glass interface. Differences in the electrical characteristics of the two systems were not considered. Absorption of 25% of the incident microwaves is, therefore, an approximation. The single-event radiation formula is used since each quantum of microwave energy is absorbed independently of other quanta.

$I_t/I_o = e^{-\alpha x}$, where I_t is the energy transmitted through the sample;

I_o is the energy incident on the sample;

α is a constant which is specific for each experiment;

x is the path length of the incident wave.

Making the assumptions given above the following calculation can be made:

Absorbing 25% is equivalent to transmitting 75%, or

$$75/100 = e^{-\alpha l};$$

thus, $\alpha = 0.29$ per cm for water.

In this work 10^{10} ergs per cm^2 incident were applied to the spores over various time intervals. A spore is about 1μ in diameter; this gives

$$(10^{10} \text{ ergs/cm}^2) \,/\, (10^8 \mu/\text{cm}^2) = 10^2 \text{ ergs}/\mu^2 = 100 \text{ ergs}$$

incident per bacterial spore over the course of the irradiation. The relative humidity inside the target housing was estimated to be 10%, thus, it was estimated that about 10% of the spore is water. The volume of a spore is $1\mu^3$, also, one water molecule occupies about 1Å^3. The volume occupied by water in each spore is 10^{11} Å^3 since 10% of the spore volume is 10% of the 10^{12} Å, therefore, there are 10^{11} water molecules in each spore. To find the energy incident on each water molecule the energy

incident per spore is divided by the number of water molecules per spore, as follows:

(100 ergs incident per spore) / (10^{11} H_2O per spore) =

10^{-9} ergs / H_2O molecule, incident.

The approximation is now

$$I_t / (10^{10} \text{ ergs/cm}^2) = e^{-(0.29)(10^{-4} \text{ cm})}$$

taking the natural logarithm of both sides of the equation

$$\ln(I_t/10^{10} \text{ ergs per cm}^2) = -(0.29)(10^{-4} \text{ cm}),$$

$I_t = 0.99999900$ (10^{10} ergs per cm^2) and

$I_t = 9.999990 \times 10^9$ ergs/cm^2 which is the energy NOT absorbed.

To find the actual energy absorbed per bacterial spore, the energy incident is multiplied by the fraction of energy absorbed (determined above):

(0.000010)(100 ergs/spore) = 10^{-4} ergs, which is the energy absorbed by each microorganism.

The heat capacity of water in the range of physiological temperatures is very close to 4.17 Joules/gram/°C

$$\frac{1}{4.17} \text{ °C} \Big/ \left(\frac{\text{Joules}}{\text{gram}}\right)$$

Total energy incident per spore = 10^{-4} ergs or 10^{-11} Joules. Mass of the water in one spore is

(10^{11} H_2O molecules/spore)(18 a.m.u.)(1.6734×10^{-24} g/a.m.u.)

which is

3×10^{-12} grams of H_2O per spore.

$$\left[\frac{1}{4.17} \text{ °C} \Big/ \left(\frac{\text{Joules}}{\text{gram}}\right)\right] \quad \left[\frac{\text{Joules}}{\text{gram}}\right] = \text{maximum temperature rise.}$$

$$\left[\frac{1}{4.17}\ ^{\circ}C/\frac{\text{Joules}}{\text{gram}}\right]\left[\frac{10^{-11}\ \text{Joules}}{3 \times 10^{-12}\ \text{grams}}\right] = \frac{1}{4.17}\ ^{\circ}C/\frac{10^{-11}}{3 \times 10^{-12}}$$

which simplifies to

$$\frac{1}{4.17} \times 10^{-1}\ ^{\circ}C \text{ per spore} \quad \text{or about} \quad 0.8^{\circ}C.$$

This is the maximum possible temperature rise per bacterial spore, and is not sufficient to initiate an unusual physiological response. If the spore was assumed to be 100% water, the maximum possible temperature rise would be on the order of one magnitude greater, which is still less than $10^{\circ}C$.

APPENDIX B

Statistical Analysis

The method of least squares analysis of data was used. The analysis involves solving two normal equations to find the values for the estimating equation $Y = a + bx$. The two normal equations are as follows:

$$\text{I} \quad \Sigma Y = Na + b\Sigma X$$

$$\text{II} \quad \Sigma XY = a\Sigma X + b\Sigma X^2$$

where

N = number of observations

Y = percent survival

X = dose in ergs/cm^2 x 10^{-10}

a = the Y intercept, the value where the estimating equation intersects the Y axis when X is zero

b = the slope of the estimating equation; dY/dX.

The estimating equation is fitted by least squares, a procedure that minimizes the sum of the squares of the vertical deviations from the estimating equation.

The correlation coefficient, r, is a measure of the degree of variation of the observations. The closer the data points are to the graph of the estimating equation, the better the correlation coefficient. A maximum of r = 1.0 is achieved if all data points fall on the graph of the estimating equation.

$$r = \left(\frac{(\Sigma xy)^2}{(\Sigma x^2)(\Sigma y^2)} \right)^{\frac{1}{2}}$$

where

$x = (X - \overline{X})$

$y = (Y - \overline{Y})$

For the normal equations

I $\quad \Sigma Y = Na + b\Sigma X$

II $\quad \Sigma XY = a\Sigma X + b\Sigma X^2$

$\Sigma Y = 1735.500$

$\Sigma X = 69.000$

$\Sigma XY = 6812.8590$

$\Sigma X^2 = 433.7534$

Substituting and solving for a, b:

$b = 0.001166$

$a = 95.852$

For the correlation coefficient

$$r = \left(\frac{(\Sigma xy)^2}{(\Sigma x^2)(\Sigma y^2)} \right)^{\frac{1}{2}}$$

$(\Sigma xy) = 160.1724$

$\Sigma x^2 = 169.2557$

$\Sigma y^2 = 363.4007$

Substitution and solving for r

$r = 0.6458$, which indicates that the data are not randomly distributed.

N, the number of observations = 18

The variance, S^2

$$S^2 = \frac{\Sigma X^2}{N} - \left(\frac{\Sigma X}{N}\right)^2$$

$$S^2 = 21.33$$

The standard deviation, S:

$$S = \sqrt{S^2} = 4.62$$

The standard error of the mean, $\sigma_{\bar{x}}$:

$$\sigma_{\bar{x}} = \frac{S}{\sqrt{N}} = 1.09$$

If the slope of the regression line, b, is not significantly different from zero, there is no significant effect of microwave dose on the survival of the bacterial spores. In order to test the significance of b, the student's t-test was used.

$$t = b\sqrt{\frac{(n-2)(\Sigma x^2)}{\Sigma y_s^2}} \qquad n = N - 2 \text{ degrees of freedom}$$

$\Sigma x^2 = 169.2557$

$\Sigma y^2 = 363.4007$

$N-2 = 16$

$b = 0.0012$

$$t = 0.0012\sqrt{\frac{(16)(169.2557)}{363.4007}} = \sqrt{\frac{2708.09120}{363.4007}}\left(0.0012\right) =$$

$$(7.45208)(0.0012) = 0.00894 = t.$$

This value of t is far above the 0.9 level and the conclusion is that b is not significantly different from zero.

Since the correlation coefficient suggests that the data points are not randomly distributed, and the t-test suggests that b is not significantly different from zero, it is concluded that there is no significant change in survivability of the bacterial spores over the range of microwave power densities tested.

APPENDIX C

Trypticase Soy Broth

	Grams/liter
Trypticase peptone	17.0
Phytone peptone	3.0
Sodium chloride	5.0
Dipotassium phosphate	2.5
Dextrose	2.5

Suspend 30 grams of the above mixture in one liter distilled water and mix while warming gently until it boils. Autoclave at 121°C (15 pounds pressure) for fifteen minutes.

Trypticase Soy Agar

	Grams/liter
Trypticase peptone	15.0
Phytone peptone	5.0
Sodium chloride	5.0
Agar	15.0

Suspend 40 grams of the above mixture in one liter of distilled water and mix while warming gently until it boils. Autoclave at 121°C (15 pounds pressure) for fifteen minutes.

Trypticase Soy Agar and Trypticase Soy Broth are trademarks of BBL, Division of Bioquest, Cockeysville, Maryland, 21030.

LITERATURE CITED

(1) L. B. Hall, J. R. Miles, C. W. Bruch, and P. Tarver, The Objectives and Technology of Spacecraft Sterilization. (In) American Astronautical Society Symposium on Unmanned Exploration of the Solar System, Proceedings, Denver, Colorado, 8-10 February 1965. NASA, Washington, D. C.

(2) D. M. Portner, Investigation of Microbial Contamination Inside Irradiated and Heated Electronic Components. Physical Defense Division, Fort Detrick, Md., Unpubl. Prelim. Data, 20 April 1965.

(3) C. W. Bruch, M. G. Koestenen, and M. R. Bruch, Dry Heat Sterilization: Its Development and Application to Components of Exobiological Space Probes. 19th Mtg. Soc. Indust. Micro., Amer. Inst. Biol. Sci., Oregon State Univ., Corvallis, Oregon, 26-31 August 1962. NASA, Washington, D. C.

(4) H. Levinson and M. Hyatt, Some Effects of Heat and Ionizing Radiation on Spores of Bacillus megaterium. J. Bact. 80:441-451, 1960.

(5) O. Rahn, Physical Methods of Sterilization of Microorganisms. Bact. Rev. 9:1-47, 1945.

(6) I. L. Shechmeister, Sterilization by Ultraviolet Radiation. (In) Disinfection, Sterilization, and Preservation, pp. 761-777, C. Lawrence and S. Block (Ed.), Lea and Febiger Publ., Philadelphia, 1968.

(7) M. T. Carpendale, Comparison of the Bactericidal Effect of Visible Light with Ultraviolet Light on Staphylococcus aureus. Physics in Med. and Biol. 41:575-579, December 1960.

(8) C. H. Addington, C. Osborn, G. Schwartz, F. P. Fischer, R. A. Newbauer, and Y. T. Sarkees, Biological Effects of Microwave Energy at 200 Mc. (In) Tri-Service Conference on the Biological Effects of Microwave Radiation, Proceedings, Volume 1, pp. 177-186, 16-18 August 1960, M. F. Peyton (Ed.), Plenum Press, New York, 1961.

(9) A. Anne, M. Saito, O. M. Salati, and H. P. Schwan, Relative Microwave Absorption in Cross Sections of Biological Significance. (In) Tri-Service Conference on the Biological Effects of Microwave Radiation, Proceedings, Volume 1, pp. 153-176, 16-18 August 1960, M. F. Peyton (Ed.), Plenum Press, New York, 1961.

(10) S. A. Goldblith, Basic Principles of Microwaves and Recent Developments, Adv. Food Tech. 15:277-301, 1966.

(11) J. E. Boysen, A Review of Unanswered Biological Hazard Operational Problems. (In) Tri-Service Conference on the Biological Effects of Microwave Radiation, Proceedings, Volume 1, pp. 309-317, 16-18 August 1960, M. F. Peyton (Ed.), Plenum Press, New York, 1961.

(12) R. V. Lechowitz, L. R. Beuchat, K. I. Fox, and F. H. Webster, Procedure for Evaluating the Effects of 2450 Megahertz Microwaves upon Streptococcus faecalis and Saccharomyces cerevesiae. Appl. Microbiol. 17(1):106-110, 1969.

(13) S. A. Goldblith and D. I. C. Wang, Effect of Microwaves on E. coli and Bacillus subtilis. Appl. Microbiol. 15(6):1371-1375, November 1967.

(14) V. T. Tomberg, Specific Thermal Effects of High Frequency Fields. (In) Tri-Service Conference on the Biological Effects of Microwave Radiation, Proceedings, Volume 1, pp. 221-228, 16-18 August 1960, M. F. Peyton (Ed.), Plenum Press, New York, 1961.

(15) G. J. Silverman, Survey of Certain Nonthermal Methods of Decontamination and Sterilization. (In) NASA Publication SP-108, pp. 193-206, 1965.

(16) J. Fleming, Jr., L. Pinneo, R. Baus, Jr., and R. McAfee, Microwave Radiation in Relation to Biological Systems and Neural Activity. (In) Tri-Service Conference on the Biological Effects of Microwave Radiation, Proceedings, Volume 1, pp. 229-249, 16-18 August 1960, M. F. Peyton (Ed.), Plenum Press, New York, 1961.

(17) R. D. McAfee, C. Berger, and P. Pizzolato, Neurological Effect of 3 cm Microwave Irradiation. (In) Tri-Service Conference on the Biological Effects of Microwave Radiation, Proceedings, Volume 1, pp. 251-259, 16-18 August 1960, M. F. Peyton (Ed.), Plenum Press, New York, 1961.

(18) J. E. Boysen, USAF Experience with Microwave Exposure. J. Occup. Med. 4:196, April 1962.

(19) J. E. Boysen, Hyperthermic and Pathologic Effects of Electromagnetic Radiation (350 Mc). AMA Arch. Ind. Hyg. 7:516, 1953.

(20) M. Lubin, G. W. Curtis, R. H. Dudley, L. E. Bird, P. F. Daley, D. G. Cogan, and S. J. Fricker, Effects of Ultrahigh Frequency Radiation on Animals. AMA Arch. Ind. Hyg. 21:555-558, June 1960.

(21) J. C. Lewis, Dormancy. (In) The Bacterial Spore, G. W. Gould and A. Hurst (Ed.), Academic Press, New York, 1969.

(22) R. F. Boggs, Determination of the Effects of Electromagnetic Energies on the Hematologic System, Ph.D. Thesis, Georgia Institute of Technology, Atlanta, Georgia, 1971.

(23) D. A. Copson, Microwave Heating, Avi Publ. Co., Inc., Westport, Conn., 1962.

(24) International Telephone and Telegraph Company, Reference Data for Radio Engineers, Fourth Edition, p. 749, New York, 1967.